ZOOLOGICAL SCIENCE 6: 345–350 (1989)

Pituitary Hormone-Dependent Aldosterone Secretion in *Xenopus laevis*

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ABSTRACT—Involvement of pituitary hormones in aldosterone secretion in *Xenopus laevis* was investigated. Both hypophysectomy and distalobectomy reduced plasma aldosterone concentration. However, the aldosterone levels were higher in the animals retaining neurointermediate lobe than those lacking total hypophysis. Neurointermediate lobe homogenate was as potent as anterior lobe homogenate in elevating aldosterone levels in hypophysectomized toads. Administration of adrenocorticotropic hormone (ACTH) to intact or distalobectomized animals increased plasma concentrations of aldosterone dose-dependently. Response to ACTH was smaller in distalobectomized animals than in intact ones. α-Melanocyte-stimulating hormone did not affect aldosterone levels in hypophysectomized toads. Both arginine vasotocin (AVT) and mesotocin (MT) were effective in elevating aldosterone levels in hypophysectomized animals, AVT being more potent than MT. The pituitary gland of *X. laevis* seems to contain multiple principles which affect aldosterone secretion.

INTRODUCTION

It has been reported that renin-angiotensin system is operating to regulate aldosterone secretion in amphibians as in other vertebrates [1]. However, aldosterone secretion seems to be also under pituitary control. Many investigators have observed that adrenocorticotropic hormone (ACTH) stimulates aldosterone secretion in anurans [2–8] and urodeles [9, 10]. On the other hand, the evidence has accumulated that in mammals non-ACTH peptides of pituitary origin have an aldosterone-releasing activity [11–14].

This study was designed 1) to confirm the effect of ACTH on the release of aldosterone, 2) to compare the effect of removal of the total hypophysis with that of removal of the anterior lobe on aldosterone secretion and 3) to examine the aldosterone-releasing activity of anterior and neurointermediate lobes as well as that of main neurointermediate lobe hormones, namely α-melanocyte-stimulating hormone (α-MSH) and arginine vasotocin (AVT) and mesotocin (MT), using clawed toads, *Xenopus laevis* juveniles.

Accepted July 14, 1988
Received June 20, 1988

MATERIALS AND METHODS

Animals

Xenopus laevis juveniles weighing about 8 g were used. They were kept under an artificial daylight regime getting illumination from 8:00 to 20:00 hr each day and were maintained at 22±2°C. They were fed every other day, the water being changed prior to the feeding.

Hypophysectomy

The toads were anesthetized by immersion in the water cotaining MS 222. Hypophysectomy was performed by a transpalatine approach. After the epithelial covering of the palate was cut and retracted, a U-shape cut was made through sphenoid bone by a small surgical knife and the flap of the bone was deflected, the brain-pituitary region being exposed. Either the whole pituitary or the anterior pituitary was taken out with fine forceps.

Administration of hormones

Porcine ACTH and α-MSH were purchased from Sigma. AVT and MT were obtained from Bachem. Each hormone was dissolved in frog Ringer's solution. 50 μl of the solution was injected into the dorsal lymph sac. All injections were performed at 9–11 a.m. Anterior and neurointermediate lobes from *X. laevis* or *Rana catesbeiana* were separately homogenized in frog Ringer's solution. The homogenate was also injected as described above.

Collection of plasma samples

Blood was collected into heparinized glass tubes by heart puncture. Collection was finished within 2 min to avoid elevation of aldosterone levels due to blood taking [15].

Aldosterone radioimmunoassay

Aldosterone in each plasma sample (50 μl) was extracted with 1.0 ml of methylene chloride. 800 μl of the methylene chloride layer was transferred to a glass tube and evaporated by a stream of nitrogen gas. The evaporated samples were diluted in 1.0 ml of 0.05 M potassium phosphate buffer (pH 7.4) containing 0.15 M NaCl, 0.1% NaN_3, and 0.5% BSA (diluent). The aliquot (0.2 ml) was mixed with 0.1 ml of antialdosterone serum (Miles-Yeda) which had been reconstituted and diluted 1:7 with diluent. The cross reactivity of various steroids with the antibody was below 0.1%. After incubation for 30 min at room temperature, 8,000 dpm of ^{3}H-aldosterone (New England Nuclear Co., 72 Ci/mmol) in 50 μl of diluent was added and the tubes were incubated at 4°C overnight. After incubation, 0.1 ml dextran-coated charcoal (0.1% dextran T-70, 0.5% charcoal in diluent) was added and samples were mixed on a Vortex mixer. The radioactivity of an aliquot (0.25 ml) of the supernatant was counted in scintillant (1 liter of Triton-X100, 2 liters of toluene, 12 g PPO, 600 mg POPOP). The intraassay coefficient of variation was 9% and interassay coefficient of variation was 10%. The sensitivity of the assay defined as twice the standard deviation at zero dose was 6.7 pg/tube.

RESULTS

As illustrated in Figure 1, complete decline of aldosterone levels was observed by 24 hr after hypophysectomy. Effects of hypophysectomy and of distalobectomy on aldosterone levels were studied. Both hypophysectomized and distalobectomized animlas exhibited low concentrations of plasma aldosterone when measured 5 days after operation. In the totally hypophysectomized animals, however, aldosterone levels were lower

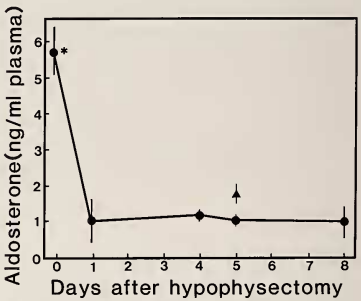


Fig. 1. Effect of hypophysectomy on plasma aldosterone levels in *X. laevis*. The value for totally hypophysectomized animals (●) is significantly different from the value for distalobectomized ones (▲) when compared 5 days after operation ($P<0.01$, Student's *t*-test). Each point and vertical line represent mean of 5 determinations and SEM, respectively. *The value is significantly different from other values at 1% level (analysis of variance).

than in the distalobectomized ones (Fig. 1). Effect of ACTH on aldosterone levels in distalobectomized and intact animals was studied. In both cases, aldosterone peak was seen 20 min after injection. In distalobectomized animals, the levels became as low as the initial levels when 24 hr elapsed after injection (Fig. 2). As shown in Figure 3, aldosterone levels elevated according to the amount of ACTH injected. It was revealed that responsiveness to ACTH was smaller in distalobectomized animals than in intact ones. Treatment with homogenate of neurointermediate lobe as well as homogenate of the anterior lobe from

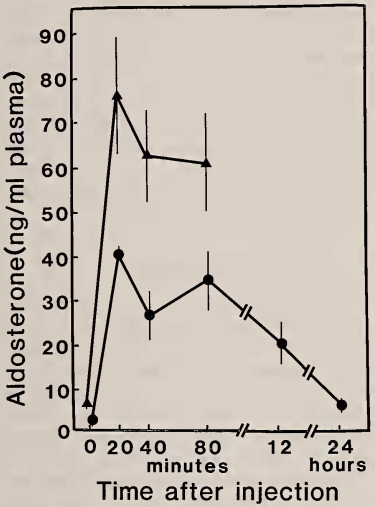
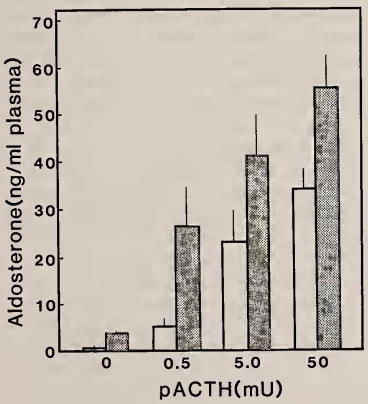


FIG. 2. Time course of response to ACTH. Distalobectomized (●) or intact (▲) animals received 50 mU ACTH. At the indicated time after injection, plasma samples were prepared for aldosterone assay. Each point and vertical line represent mean of 5 determinations and SEM, respectively.



bullfrogs and *X. laevis* resulted in the elevation of aldosterone levels to a similar extent (Fig. 4). In order to ascertain whether α-MSH, AVT, and MT have any influence upon aldosterone secretion, these hormones were separately injected to hypophysectomized animals. α-MSH (250 ng) showed little effect during the period of 20–80 min after injection (Fig. 5). Neither lower nor higher

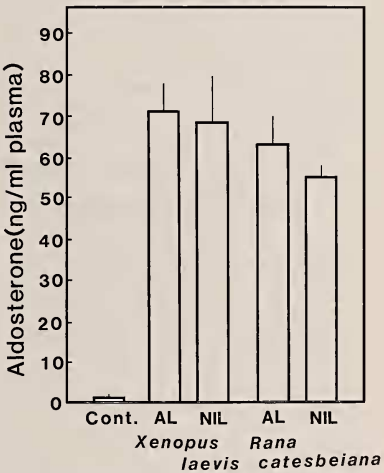


FIG. 4. Effect of pituitary homogenate on aldosterone levels in hypophysectomized *X. laevis*. Test samples were obtained from anterior lobes (AL) and neurointermediate lobes (NIL) of *X. laevis* and *Rana catesbeiana*. Each homogenate injected contained 25 μg protein. Each column and vertical bar represent mean of 5 determinations and SEM, respectively. The values for treated groups are significantly different from the value for controls at 1% level (analysis of variance).

FIG. 3. Effect of ACTH on plasma aldosterone levels in intact and distalobectomized *X. laevis*. Various doses of ACTH as indicated were given to the animals. Blood samples were collected 20 min after injection. Each column and vertical bar represent mean of 5 determinations and SEM, respectively. The value for intact animals is significantly different from the value for distalobectomized animals treated similarly at 1% level (Student's *t*-test).

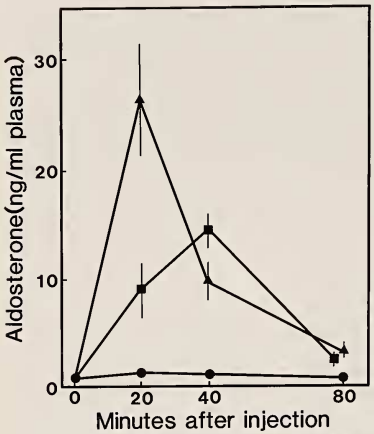


FIG. 5. Time course of response to neurointermediate lobe hormones. Hypophysectomized *X. laevis* received 250 ng α-MSH (●), 14 ng AVT (▲) and 140 ng MT (■). Blood samples were collected at the indicated time after injection. Each point and vertical line represent mean of 5 determinations and SEM, respectively.

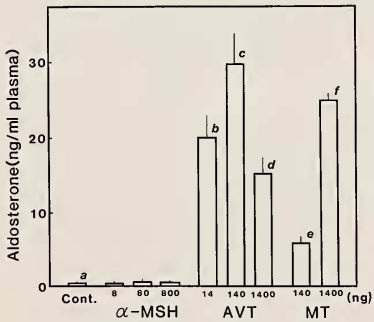


FIG. 6. Effect of neurointermediate lobe hormones on aldosterone levels in hypophysectomized *X. laevis*. Animals received injection of Ringer's solution, α-MSH, AVT or MT. Blood samples were prepared 20 min after injection. Each column and vertical bar represent mean of 5 determination and SEM, respectively. Significance of difference: a vs b, a vs c, a vs d, a vs f, c vs d, and e vs f, $P<0.01$ (analysis of variance).

dose of α-MSH affected aldosterone levels as determined 20 min after injection (Fig. 6). On the other hand, both AVT and MT elevated aldosterone levels markedly. The peak was observed 20 min after injection in the case of AVT and 40 min after injection in the case of MT (Fig. 5). The aldosterone-releasing activity of AVT was more prominent than that of MT. In the case of AVT, the largest dose (1400 ng) tested was less effective than a smaller dose (140 ng) (Fig. 6).

DISCUSSION

It was confirmed that ACTH is a powerful aldosterone-release stimulator in *X. laevis*. In the intact animal, ACTH produced quantitatively greater response of interrenals than the same hormone when administrated to the animals lacking the anterior lobe. In the iguanid lizard, Daugherty and Callard [16] observed that in hypophysectomized animals, the adrenal response to ACTH is slow and small as compared with the response in intact animals. They assumed that the poor response in hypophysectomized specimens is due to the decline of the entire level of steroidogenic activity which is dependent on ACTH and the absence of other pituitary hormones to act synergistically with ACTH.

In the present experiment, aldosterone levels were markedly lowered by the removal of the distal lobe of the pituitary gland as well as the removal of the total pituitary gland, the levels being lower in the case of removal of the total hypophysis than in the case of removal of the anterior lobe only. This indicates that some hormonal factor(s) released from the neurointermediate lobe has a stimulatory, though minor, effect on aldosterone release. According to Leboulenger *et al.* [17], there was no significant difference in corticosterone levels between totally hypophysectomized and distalobectomized frogs (*Rana esculenta*). It was demonstrated that the neurointermediate lobe homogenate was as potent as the anterior lobe homogenate in elevating plasma aldosterone levels. Delarue *et al.* [3] have also reported that the intermediate lobe homogenate caused a considerable increase in aldosterone release from interrenal fragments of *Rana ridibunda*

in vitro. They assumed that ACTH which is detectable in pars intermedia by means of radioimmunoassay [18] is responsible for the elevation of aldosterone secretion. Recently, Leboulenger *et al.* [19] have reported that *Rana ridibunda* interrenals respond to α-MSH, desacetyl α-MSH and γ-MSH to release corticoids *in vitro*. In the present experiment, α-MSH exhibited no aldosterone-releasing activity in *Xenopus*. On the other hand, AVT increased aldosterone levels markedly. Hanke and Masor [20] have observed that AVT stimulates the *in vitro* release of both aldosterone and corticosterone from the interrenal tissue according to the concentrations (1-100 ng/ml), suggesting that the posterior lobe hormone acts directly on the interrenal tissue. According to our data, higher dose (1400 ng) of AVT was not so effective as the lower one (140 ng). This might happen if AVT acts *in vivo* to enhance aldosterone release on one hand directly and suppress on the other indirectly, the effective dose-range for each action being different. In fact, it is known that administration of vasopressin in human and several animal species suppresses renin secretion, consequently inhibiting aldosterone secretion [21]. In the present experiment, it was found that another neurohypophyseal hormone, MT is also effective in increasing aldosterone levels in hypophysectomized toads. We have tested only α-MSH and two neurohypophyseal hormones for their aldosterone-releasing activity so far. It is a matter of further investigation to separate aldosterone-releasing principles from the neurointermediate lobe as well as the anterior lobe of the amphibian pituitary gland and to clarify their mode of action.

ACKNOWLEDGMENTS

This study was supported by Grants-in-Aid from the Ministry of Education, Science and Culture, Japan and a research grant from Waseda University to S.K.

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